



# **NADP-Malate Dehydrogenase Microplate Assay Kit User Manual**

**Catalog # FTA0133**

(Version 1.2A)

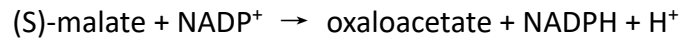
Detection and Quantification of NADP-Malate Dehydrogenase  
Activity in Serum, Plasma, Tissue extracts, Cell lysate, Cell culture  
media and Other biological fluids Samples.

**For research use only. Not for diagnostic or therapeutic procedures.**

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## I. INTRODUCTION

In enzymology, a malate dehydrogenase (NADP<sup>+</sup>) (EC 1.1.1.82) is an enzyme that catalyzes the chemical reaction



Thus, the two substrates of this enzyme are (S)-malate and NADP<sup>+</sup>, whereas its 3 products are oxaloacetate, NADPH, and H<sup>+</sup>.

This enzyme belongs to the family of oxidoreductases, specifically those acting on the CH-OH group of donor with NAD<sup>+</sup> or NADP<sup>+</sup> as acceptor. The systematic name of this enzyme class is (S)-malate:NADP<sup>+</sup> oxidoreductase. Other names in common use include NADP<sup>+</sup>-malic enzyme, NADP<sup>+</sup>-malate dehydrogenase, malic dehydrogenase (nicotinamide adenine dinucleotide phosphate), malate NADP<sup>+</sup> dehydrogenase, NADP<sup>+</sup> malate dehydrogenase, NADP<sup>+</sup>-linked malate dehydrogenase, and malate dehydrogenase (NADP<sup>+</sup>). This enzyme participates in pyruvate metabolism and carbon fixation.

NADP-Malate Dehydrogenase Assay Kit is a sensitive assay for determining NADP-Malate Dehydrogenase activity in various samples. NADP-Malate Dehydrogenase activity is determined by NADPH decomposition rate. The reaction products can be measured at a colorimetric readout at 340 nm.

## II. KIT COMPONENTS

| Component          | Volume     | Storage |
|--------------------|------------|---------|
| 96-Well Microplate | 1 plate    |         |
| Assay Buffer       | 30 ml x 4  | 4 °C    |
| Reaction Buffer    | 20 ml x 1  | 4 °C    |
| Substrate          | Powder x 1 | -20 °C  |
| Standard           | Powder x 1 | -20 °C  |
| Technical Manual   | 1 Manual   |         |

### Note:

**Substrate:** add 19 ml Reaction Buffer to dissolve before use.

**Standard:** add 1 ml distilled water to dissolve before use; then add 0.2 ml into 0.8 ml distilled water, the concentration will be 400 µmol/L.

## III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 340 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice

#### IV. SAMPLE PREPARATION

##### 1. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay buffer for  $5 \times 10^6$  cell or bacteria, sonicate (with power 20%, sonication 3s, interval 10s, repeat 30 times); centrifuged at 10,000g 4 °C for 15 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

##### 2. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml Assay buffer on ice, centrifuged at 10,000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

##### 3. For liquid samples

Detect directly.

## V. ASSAY PROCEDURE

Warm all reagents to room temperature before use.

Add following reagents into the microplate:

| Reagent                                                                            | Sample      | Standard    | Blank       |
|------------------------------------------------------------------------------------|-------------|-------------|-------------|
| Standard                                                                           | --          | 200 $\mu$ l | --          |
| Distilled water                                                                    | --          | --          | 200 $\mu$ l |
| Substrate                                                                          | 190 $\mu$ l | --          | --          |
| Sample                                                                             | 10 $\mu$ l  | --          | --          |
| Mix, measured at 340 nm and record the absorbance of 10th second and 130th second. |             |             |             |

### Note:

- 1) Perform 2-fold serial dilutions of the top standards to make the standard curve.
- 2) For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range. If the enzyme activity is lower, please add more sample into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

## VI. CALCULATION

**Unit Definition:** One unit of NADP-Malate Dehydrogenase activity is defined as the enzyme reduces 1 nmol of NADPH per minute.

### 1. According to the protein concentration of sample

$$\begin{aligned} \text{NADP-MDH (U/mg)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (OD_{\text{Sample}(10S)} - OD_{\text{Sample}(130S)}) / (OD_{\text{Standard}} - \\ &\quad OD_{\text{Blank}}) / (V_{\text{Sample}} \times C_{\text{Protein}}) / T \\ &= 4000 \times (OD_{\text{Sample}(10S)} - OD_{\text{Sample}(130S)}) / (OD_{\text{Standard}} - OD_{\text{Blank}}) / C_{\text{Protein}} \end{aligned}$$

### 2. According to the weight of sample

$$\begin{aligned} \text{NADP-MDH (U/g)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (OD_{\text{Sample}(10S)} - OD_{\text{Sample}(130S)}) / (OD_{\text{Standard}} - \\ &\quad OD_{\text{Blank}}) / (V_{\text{Sample}} \times W / V_{\text{Assay}}) / T \\ &= 4000 \times (OD_{\text{Sample}(10S)} - OD_{\text{Sample}(130S)}) / (OD_{\text{Standard}} - OD_{\text{Blank}}) / W \end{aligned}$$

### 3. According to the quantity of cells or bacteria

$$\begin{aligned} \text{NADP-MDH (U/10}^4\text{)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (OD_{\text{Sample}(10S)} - OD_{\text{Sample}(130S)}) / (OD_{\text{Standard}} - \\ &\quad OD_{\text{Blank}}) / (V_{\text{Sample}} \times N / V_{\text{Assay}}) / T \\ &= 4000 \times (OD_{\text{Sample}(10S)} - OD_{\text{Sample}(130S)}) / (OD_{\text{Standard}} - OD_{\text{Blank}}) / N \end{aligned}$$

### 4. According to the volume of sample

$$\begin{aligned} \text{NADP-MDH (U/ml)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (OD_{\text{Sample}(10S)} - OD_{\text{Sample}(130S)}) / (OD_{\text{Standard}} - \\ &\quad OD_{\text{Blank}}) / V_{\text{Sample}} / T \\ &= 4000 \times (OD_{\text{Sample}(10S)} - OD_{\text{Sample}(130S)}) / (OD_{\text{Standard}} - OD_{\text{Blank}}) \end{aligned}$$

$C_{\text{Standard}}$ : the standard concentration, 400  $\mu\text{mol/L}$  = 400 nmol/ml;

$V_{\text{Standard}}$ : the volume of standard, 200  $\mu\text{l}$  = 0.2 ml;

$C_{\text{Protein}}$ : the protein concentration, mg/ml;

W: the weight of sample, g;

N: the quantity of cell or bacteria,  $N \times 10^4$ ;

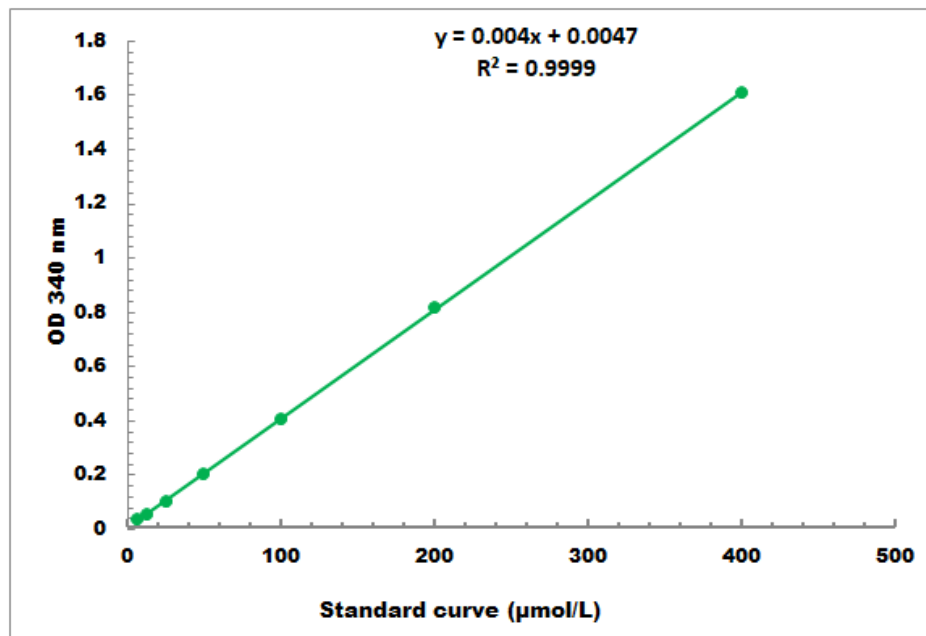
$V_{\text{Sample}}$ : the volume of sample, 0.01 ml;

$V_{\text{Assay}}$ : the volume of Assay buffer, 1 ml;

T: the reaction time, 2 minutes.

## VII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 4 μmol/L - 400 μmol/L

## VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to [www.cohesionbio.com](http://www.cohesionbio.com) or contact us at [techsupport@cohesionbio.com](mailto:techsupport@cohesionbio.com)

## IX. NOTES